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Dehydroepiandrosterone Enhances the Hypnotic and Hypothermic Effects of Ethanol and Pentobarbital

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MELCHIOR, C. L. AND R. F. RITZMANN. *Dehydroepiandrosterone enhances the hypnotic and hypothermic effects of ethanol and pentobarbital.* PHARMACOL BIOCHEM BEHAV 43(1) 223–227, 1992.—Recent reports have indicated that the neurosteroid dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) interact with the $GABA_A$ receptor complex. Because many of the behavioral effects of ethanol and pentobarbital are due to activity at this complex, DHEA and DHEAS were tested for their ability to interact with the hypnotic and hypothermic effects of ethanol and pentobarbital. DHEA, but not DHEAS, causes a dose-dependent increase in the sleep time induced by either ethanol or pentobarbital. At 20 mg/kg, DHEA and DHEAS themselves cause a fall in body temperature. DHEA enhances the hypothermic effect of both ethanol and pentobarbital. DHEAS enhances the hypothermic effect of ethanol, but with pentobarbital it only delays the return of body temperature to baseline levels. Neither DHEA nor DHEAS affects the metabolism of ethanol.

Dehydroepiandrosterone Dehydroepiandrosterone sulfate Neurosteroid Ethanol
Pentobarbital Hypnosis Hypothermia GABA

SOME steroids, including dehydroepiandrosterone (DHEA) and its sulfate (DHEAS), have recently been shown to be produced de novo in the brain and have therefore been called neurosteroids (2,5). Investigations of the neurochemical and electophysiological actions of neurosteroids have revealed that many of them are active at the $GABA_A$ receptor, acting as either allosteric agonists or antagonists (11). Studies of DHEAS have shown that the binding of this compound is inhibited by the barbiturates pentobarbital and phenobarbital, as well as the neurosteroid pregnenolone sulfate, DHEAS reduces the potency of pentobarbital to increase flunitrazepam binding, and DHEAS impedes GABA-induced currents in neurons (7,12), indicating that DHEAS interacts at the barbiturate site with the $GABA_A$ receptor as an allosteric antagonist (7,12). Although DHEA does not interact with the DHEAS binding site, electrophysiological data shows that DHEA as well as DHEAS blocks GABA-induced current in cultured neurons from ventral mesencephalon (7). In addition, both DHEA and DHEAS increased neuronal excitability when applied iontophoretically on neurons from the septopreoptic area of guinea pigs, as would be expected of compounds that block GABA function (3).

The GABA-benzodiazepine-chloride receptor complex has been shown to be important for many of the behavioral responses to ethanol (21). For example, the anesthetic effects of ethanol are enhanced by GABA mimetic agents and reduced by GABA antagonists (13). Thus, compounds that demonstrate effects such as GABA antagonist activity become logical candidates to test for possible interactions with the effects of ethanol.

The effects of DHEA and DHEAS on the behavioral actions of ethanol have not previously been examined. However, preliminary reports (6,14,16,22) indicate that oral or intraperitoneal administration of moderate doses of ethanol in rats profoundly lowers the level of DHEA plus DHEAS in the brain within 1 h after ethanol followed by a return to approximately baseline levels at 4–5 h after ethanol (6,16,22). Vatier and Bloom (22) concluded that DHEA appeared to be a marker of alcohol intoxication in the limbic system.

The purpose of the present experiments was to assess the effects of the neurosteroids DHEA and DHEAS on the hypnotic and hypothermic effects of ethanol. Interactions with these actions of pentobarbital were also evaluated for comparison.

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METHOD

Subjects

Male C57BL/6 mice weighing 22 ± 2 g, obtained from the National Cancer Institute, were used in these studies. Animals were housed in a temperature-controlled environment with a 12 L : 12 D cycle and continuous access to food and water.

Drugs

DHEA and DHEAS were obtained from Sigma Chemical Co. (St. Louis, MO). DHEA was suspended in a 0.4% (w/v) Tween-80/saline solution. This Tween/saline solution served as the vehicle control for injections of DHEA while saline was administered as a control for other injections. DHEAS was dissolved in saline. Ethanol was prepared as a 20% w/v solution of 95% ethanol. Pentobarbital was prepared by diluting Nembutal with saline. All injections were administered in a volume of 0.1 cc/10 g body weight except ethanol and the saline control for ethanol, which were administered in a volume of 0.175 cc/10 g. DHEA or DHEAS were injected immediately after either 3.5 g/kg ethanol or 50 mg/kg pentobarbital.

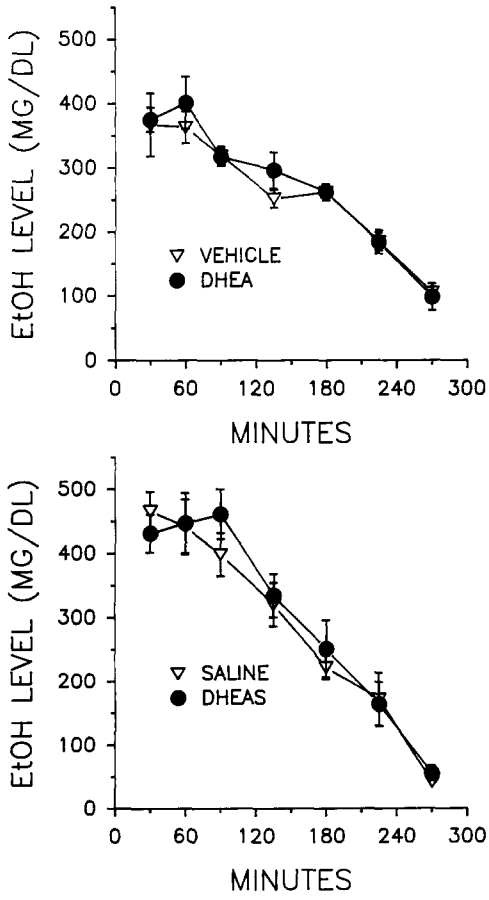

FIG. 1. BELs obtained at various times after injection of 3.5 g/kg ethanol along with vehicle or DHEA (top) or saline or DHEAS (bottom). n = 6/group.

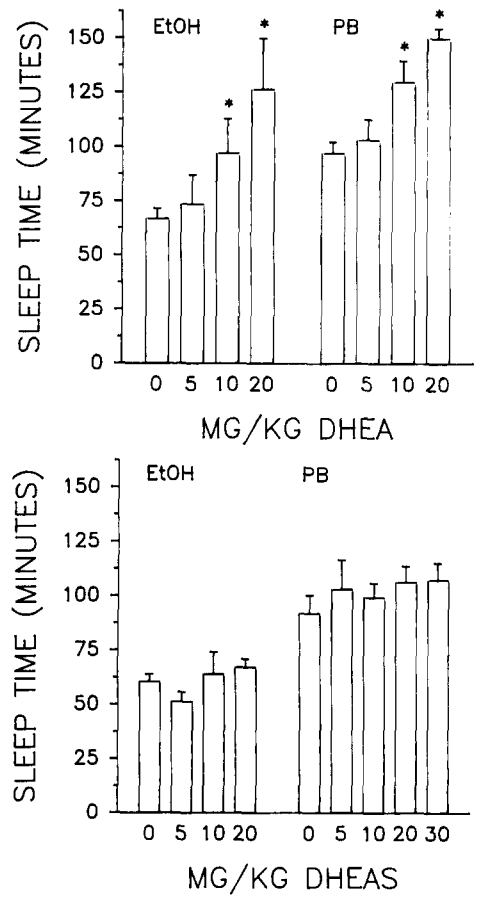

FIG. 2. Sleep time produced by 3.5 g/kg ethanol (EtOH) or 50.0 mg/kg pentobarbital (PB) in combination with various doses of DHEA (top) or DHEAS (bottom). n = 6/group. *$p < 0.05$ compared to 0 mg/kg, Duncan's range test.

Blood Ethanol Level

Blood samples (5 μl) were obtained from the tail vein at various times after injections (see Fig. 1). Blood ethanol levels (BELs) were determined by a gas chromatographic procedure as previously described (19). Briefly, samples were placed in sealed vials containing 2 ml of a solution of perchloric acid with 25 mM thiourea. The vials were heated to 55°C in a water bath. Two milliliters of the head space was injected into a gas chromatograph (GC) with a flame ionization detector and a Poropack (Alltech Associates, Inc., Deerfield, IL) Q column. Temperature settings for the GC were: detector 250, injection port 150, oven 150. The flow rates in ml/min were: nitrogen (carrier gas) 40, hydrogen 30, air 200. Under these conditions, the retention time for ethanol is approximately 4 min.

Sleep Time

After injections, animals were placed in V-shaped sleeping troughs and time until the subject was able to be placed on its back was measured. Thereafter, the time until it was able to right itself three times within 30 s was recorded. Sleep time was defined as the time from the loss of the righting reflex to the time the reflex was regained.

Body Temperature

The effect of the drug injections on body temperature was determined with a Yellow Springs Instruments Telethermometer (Yellow Springs, OH) at various times after injections. To determine body temperature, a probe lubricated with Vaseline was inserted 2.5 cm into the rectum. The thermometer was allowed 30 s to stabilize prior to reading.

Statistical Analysis

Data were analyzed by either one-way or repeated-measures analysis of variance (ANOVA). Following significant F ratios, a multiple-range test was performed. All data are expressed as mean ± SEM.

RESULTS

Neither DHEA nor DHEAS at 20 mg/kg affected the metabolism of 3.5 g/kg ethanol (Fig. 1).

As shown in Fig. 2, DHEA caused a dose-dependent increase in sleep time induced by either ethanol, $F(3, 23) = 13.05$, $p < 0.01$, or pentobarbital, $F(3, 24) = 8.91$, $p < 0.01$. In contrast, DHEAS did not influence the sleep time induced by either ethanol or pentobarbital. A lower dose of 0.5 mg/kg of either DHEA or DHEAS, in the range shown to have an effect on memory in mice (8), was also without influence on ethanol-induced sleep time.

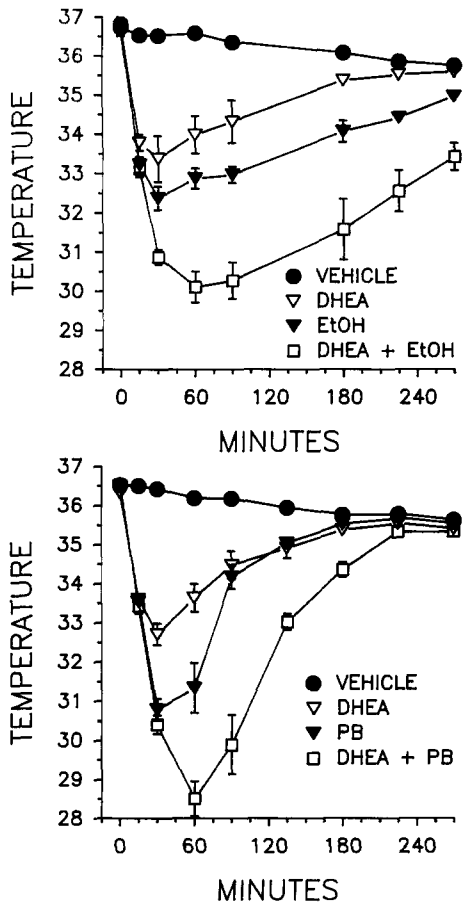

FIG. 3. Effect on body temperature (°C) of an injection of vehicle plus saline (VEHICLE), 20.0 mg/kg DHEA plus saline (DHEA), vehicle plus 3.5 g/kg ethanol (EtOH), or 20.0 mg/kg DHEA plus 3.5 g/kg ethanol (DHEA + EtOH) (top) and VEHICLE, DHEA, vehicle plus 50.0 mg/kg pentobarbital (PB), or 20.0 mg/kg DHEA plus 50.0 mg/kg pentobarbital (DHEA + PB) (bottom). $n = 6$/group.

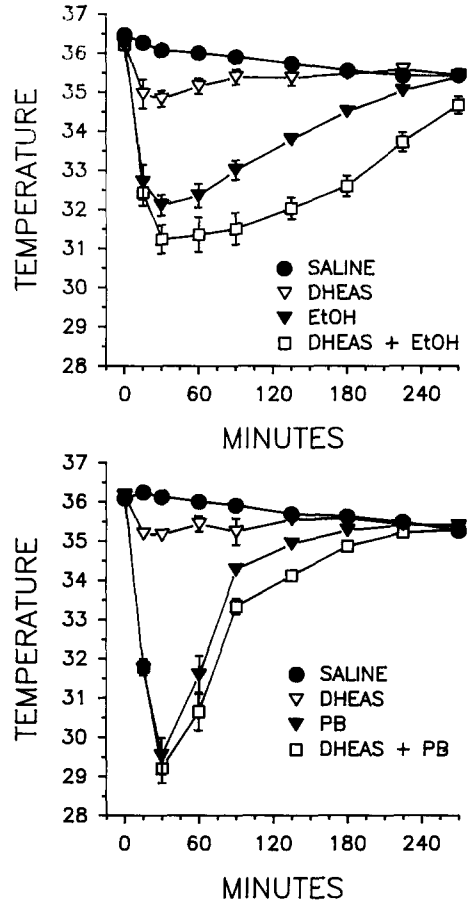

FIG. 4. Effect on body temperature (°C) of an injection of saline plus saline (SALINE), 20.0 mg/kg DHEAS plus saline (DHEAS), saline plus 3.5 g/kg ethanol (EtOH), or 20.0 mg/kg DHEAS plus 3.5 g/kg ethanol (DHEAS + EtOH) (top) and SALINE, DHEAS, saline plus 50.0 mg/kg pentobarbital (PB), or 20.0 mg/kg DHEAS plus 50.0 mg/kg pentobarbital (DHEAS + PB) (bottom). $n = 6$/group.

At 20 mg/kg, DHEA caused hypothermia. As shown in Fig. 3, DHEA significantly enhanced the hypothermia caused by ethanol or pentobarbital [treatment effect, ethanol, $F(3, 20) = 64.86$, $p < 0.01$, pentobarbital, $F(3, 20) = 78.63$, $p < 0.01$; in both experiments, all groups were significantly different from all the others].

Figure 4 shows that DHEAS at 20 mg/kg also caused hypothermia and enhanced the hypothermia produced by injections of ethanol or pentobarbital [treatment effect, ethanol, $F(3, 20) = 57.46$, $p < 0.01$, pentobarbital, $F(3, 20) = 123.31$, $p < 0.01$]. However, with pentobarbital the difference was not due to a greater maximal fall in temperature but rather to a slower return to baseline.

DISCUSSION

DHEA, but not DHEAS, enhances the hypnotic and hypothermic effects of both ethanol and pentobarbital. In the doses

tested, neither DHEA nor DHEAS alone causes the loss of righting reflex, but they both cause hypothermia. While DHEA clearly increases the hypothermic effect of both ethanol and pentobarbital, the interaction of DHEAS with the thermal effects of these drugs is characterized by a delay in return to baseline. With ethanol, but not with pentobarbital, DHEAS did enhance the maximal fall in body temperature. The observation that DHEA enhances depressant action is consistent with other studies of the behavioral actions of DHEA. Analysis of electroencephalograms (EEGs) and the behavior of monkeys given DHEA revealed dose-dependent effects of the steroid. At low doses, behavioral sedation was accompanied by high amplitude slow-wave EEG activity whereas high doses caused seizures (10). In the present study, higher doses of both DHEA and DHEAS (80 or 100 mg/kg) were not utilized because they cause fatal convulsions (unpublished observations).

An antiaggressive action of DHEA has also been documented. In mice, DHEA inhibited the aggressive behavior of castrated males toward lactating female intruders (9,23) and in rats DHEA reduced sexual performance and intermale aggression (20).

DHEA has antiobesity and anticancer actions (4,15). Investigations of the effects of DHEA have shown that, among various metabolic actions, the compound alters the activities of several liver enzymes (4,15,17). It was therefore particularly important to note in the present study that acute injection of neither DHEA nor DHEAS altered the disposition of ethanol. However, the influence of DHEA on various metabolic processes may contribute to the behavioral actions of this compound both alone and in combination with other drugs.

Although DHEA did not alter the disposition of ethanol, Robel et al. (14) reported that, following intramuscular injection of DHEA, DHEA was cleared much more rapidly from the brain in rats treated with ethanol than in controls, suggesting that ethanol causes an increased metabolic conversion of DHEA. Ethanol has been reported to increase the metabolic conversion of DHEA to 5-androsten-3β-17β-diol (1). Thus, metabolites of DHEA may contribute to the behavioral effects noted when ethanol and DHEA are both administered.

Although the hypnotic effects of ethanol can be influenced by GABA and benzodiazepine agonists and antagonists, there is little indication that the thermal effects of ethanol are mediated by the GABA-benzodiazepine-chloride receptor complex (18). Both GABA agonists and antagonists cause a fall in body temperature in mice and both also can enhance the hypothermic effect of ethanol (13). Therefore, interactions with ethanol on this measure do not allow for conclusions with respect to activity at the $GABA_A$ receptor.

Although receptor binding and electrophysiological data indicate that DHEA and DHEAS act as negative modulators of the $GABA_A$ receptor, these studies of interactions of DHEA and DHEAS with ethanol and pentobarbital suggest that the response to these compounds is not what would be expected based upon behavioral data from other GABA antagonists. There are several possible reasons for these observations. Studies of receptor binding of DHEAS and other steroids have lead to the conclusion that there may be multiple steroid regulatory sites associated with the $GABA_A$ receptor complex (7), allowing for a profile of action of these drugs at the GABA receptor that may not be analogous to that of other substances active at this receptor complex. In addition, peripheral injection allows for contributions by actions on other metabolic processes and by metabolites. Further studies are required to determine the mechanism for the interaction of these compounds with ethanol and pentobarbital.

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